FRESHWATER BIOLOGICAL ASSOCIATION

The Ferry House, Ambleside, Cumbria LA22 OLP

# NITRATES - LOSS PROCESSES IN RAW WATER

by

GRAHAME H. HALL

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| Project Leader: | G.H. Hall |
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Summary of main conclusions

1. Slight changes in the method used to prepare sediment slurries can result in large changes in the measured nitrifying activity. This makes comparisons between studies, using different methods, extremely difficult.
2. Methods to study sediment nitrification processes which do not disrupt preformed substrate gradients within the sediment provide the most reliable rate estimates.
3. On an annual basis the nitrate load to Grasmere lake from the catchment was 157 kg N $ha^{-1}$ compared to nitrate produced by nitrification within the lake of 80 kg N $ha^{-1}$. If these separate nitrate loads were calculated on a monthly basis their relative importance to the total nitrate load to the lake showed considerable variation.
4. Rates of nitrification were greatest in the surface sediments of the littoral zone. However, in terms of nitrate produced within the lake the water column had most influence as the volume which could support nitrification was 700 times greater than the active volume of sediment.
5. Increased eutrophication of lakes resulted in increased rates of nitrification in the deeper waters and surface sediments of the littoral zone. Such effects were not observed in profundal sediments possibly due to inhibition by unknown mechanisms.

# OVERVIEW

This project investigated the production of nitrate (nitrification) by bacteria in lakes. The work was undertaken as nitrification is a key process in the nitrogen cycle and previous estimates of rates of nitrification were unreliable.

When different methods were used to estimate rates of nitrification within sediment deposits different results were obtained. Investigation of specific aspects of these methodologies has allowed some rationalization of these observations and also enabled comparisons of previously published data which, beforehand, was not possible. However, it was not clear which methods gave the most reliable rate estimates. Calculation of a nitrate budget for Grasmere lake indicated that the use of methods which involved the mixing of surface sediments (and therefore disrupted preformed nutrient gradients) overestimated the rate of nitrification. We have succeeded in obtaining realistic estimates of nitrification in a range of lakes differing in degree of eutrophication. Rates of nitrification increased in both the deep water and littoral surface sediments of lakes in response to increased eutrophication. In the surface of profundal muds nitrification activity did not increase with eutrophication. Moreover, in very productive lakes nitrification appeared to be inhibited in these sediments. These data may be useful for predicting rates to be expected in other lake systems.

We have calculated the amount of nitrate produced within Grasmere lake and compared this with the amount of nitrate entering the lake from the catchment via the main inflow, the river Rothay. On a monthly basis the nitrate produced within the lake could be 7 to 40% of the amount of nitrate entering the lake via the inflow. However, the summation of these values to cover an annual cycle showed that nitrate produced within the lake was only half that entering the lake from the catchment.

Although rates of nitrification were greater in the sediments than in the water column, the volume of water capable of supporting nitrification was approximately 700 times that of the sediment. Consequently nitrification occurring in the water column had a greater impact on nitrate dynamics than nitrification within the sediments.

1. To investigate how the measured variation in rates of nitrification in aquatic sediments is affected by different sampling methods.
2. By sediment coring and micro-coring techniques to determine the dynamics of nitrification in sediments. To establish the relative importance of in situ and catchment derived nitrate fluxes.
3. To undertake the rate measurements of item 2 in sediments of water bodies exhibiting differing characteristics and qualities.
4. To examine unusual patterns of nitrification which appear to be associated with the re-introduction of oxygen to the reduced sediments of eutrophic lakes upon lake overturn. Sampling to be undertaken in additional lakes to help with this exercise.

## General Introduction

Nitrogen has been recognised as one of the major nutrients involved in the eutrophication of surface waters. Consequently much attention has been given to bacterial transformations which represent sources and sinks of nitrogen to lake ecosystems. Accordingly previous work at this laboratory, some under contract to DoE, has attempted to quantify the denitrification process in which nitrate is reduced to gaseous end products which can subsequently be lost from the lake. This work identified that a major gap in our understanding of the nitrogen cycle in lakes was of the process of nitrate production or nitrification. This process represents the oxidation of ammonia to nitrite which is subsequently oxidised further to nitrate. Each oxidation is mediated by separate specialized groups of bacteria. The oxidation of ammonia or nitrite provides electrons for energy production. Recent biochemical studies have indicated that only 1 mole of ATP is formed per mole of substrate oxidised and consequently a rapid turnover of nitrogen can occur without correspondingly large increases in biomass. The potential for rapid nitrogen turnover means that nitrification is probably the most important link between reduced and oxidised sides of the nitrogen cycle and is therefore of fundamental importance in the nitrogen budget of all ecosystems.

Moreover, concern has been expressed regarding increased concentrations of nitrate in surface waters. Whilst much of this can be attributed to the increased use of nitrogenous fertilizers, there are no data on the relative importance of nitrate loading from the catchment and nitrate production within the lake. The present research program was designed to obtain a better quantitative description of nitrate production within lakes by assessing spatial distribution patterns and the effects of eutrophication on these distributions.

## Materials and methods

### Study site

The major part of this work was performed on Grasmere, a small lake measuring only 1.6 km by 0.9 km with a maximum depth of 21.5 m and mean depth of 7.7 m. The lake is subject to morphometric eutrophication and during summer the water column can totally deoxygenate below 6 m depth. An analysis of lake volume and rainfall within the catchment estimated a retention time of 36 ± 28 days over a yearly cycle. Grasmere has been classified as a mesotrophic lake (Hall et al., 1978) but midway through this contract a sewage effluent, originally discharged into the main inflow (the river Rothay), was diverted to be discharged at a depth of 7 m in the main basin of the lake due north of site A (see Figure 1). Values of the hypolimnetic areal oxygen deficit (see Table 1) indicate that the lake should now be classed as eutrophic. However, the surface waters of the lake have a phytoplankton assemblage characteristic of a mesotrophic system due to short hydraulic retention times and the lack of influence of the sewage discharge. The surface water and shallow water sediments are therefore still characteristic of a mesotrophic system. Samples were obtained from sites A and D shown in Figure 1.

A number of other lakes were sampled for comparative purposes. The basic limnological features of these lakes are shown in Table 1.

### Sampling

#### Water

Water samples were taken with a one litre Friedinger water bottle and immediately transferred to clean, sterile 250 ml screw capped bottles. During detailed studies on Grasmere water samples were obtained at 0 m, 5 m, 10m, 15 m and 20 m depths from site A (Figure 1). For inter-lake comparisons all water samples were obtained from the deepest point of the lake. A depth of 5 m was taken as being representative of surface waters whilst a representative depth

of hypolimnetic water was more difficult to define. The sediments of a lake have a great influence on the water column and therefore in lakes of different depths a constant distance from the sediments was considered to be more representative than a constant depth. Therefore hypolimnetic water samples were taken from 6 m above the sediments in each lake.

Sediments

All sediment samples were taken with a Jenkin surface sediment sampler and transported back to the laboratory under subdued light conditions. Experimental procedures were started within three hours of sampling. In all the lakes sampled, sediments obtained at the deepest point were considered to be representative of profundal muds whilst sediments covered by 5 m of water were considered to be littoral muds. At least 3, up to a maximum of six, cores were taken from each site on each visit. The sediment cores were extruded using the procedures described by Ohnstad and Jones (1982).

Nitrification rate estimate

Estimates of the rates of nitrification were made in all water and sediment samples. Water samples were incubated at 20°C on a reciprocating shaker at 150 rpm. Samples for inorganic nitrogen analysis were removed at time zero and then at varying times not exceeding 18 h. Another estimate of the rate of nitrification in hypolimnetic water samples was obtained from the increase of nitrate concentrations at the same water depth between sampling trips (see Hall, 1982).

Two basic methods were used to estimate rates of nitrification in the surface 1.0 cm sediments:-

(a) Slurry technique: This technique was applied in open and closed systems.

1. Open systems: The surface 1.0 cm of sediment was removed and diluted $10^{-1}$ (w/v) with the overlying water. Ammonia was added as substrate (as $(NH_4)_2SO_4$) to a final concentration of 10 mg N $l^{-1}$ and incubation continued on an orbital incubator (220 rpm) at 20°C for times not exceeding

20 h. Occasionally additions of sodium nitrate or nitrite were made instead of ammonium sulphate. Samples for inorganic N analysis were removed at time zero and at the end of incubation. Rates of nitrification were calculated from increases in the nitrite and/or nitrate concentrations and presented on a dry wt. basis. On one occasion additional slurries were prepared and duplicate flasks incubated at 5, 10 and 15°C with comparable rates of shaking. The values for the rate of nitrification at different temperatures allowed calculation of a factor to convert the rates estimated at 20°C to the values expected at field temperatures. Variations in this basic technique were obtained by changing (a) the solution used to dilute the sediments, (b) the dilution factor of the sediment slurry, (c) the amount of substrate ($(NH_4)_2SO_4$) added and (d) the illumination of the samples. Unless stated otherwise all measurements using sediment slurries were made using these open systems.

2. Closed systems: The surface 1.0 cm of sediment was removed and diluted $10^{-1}$ (w/v) with the overlying water. Ammonia was added to a concentration of 10 mg N $l^{-1}$ (as $(NH_4)_2SO_4$) and aliquots dispensed into 125 ml serum vials and sealed using neoprene septa. These samples were degassed with Helium for 60 to 90 minutes and oxygen subsequently injected to give a head space concentration equivalent to ambient concentrations (19%). These vials were incubated at 20°C on a reciprocating shaker at 220 rpm and samples for inorganic N analysis removed at time zero and after incubation. In addition the nitrogen gas content of the headspace of the vial was determined at similar times. Preliminary experiments had indicated that nitrate production by sediment slurries was similar in the closed and open slurry systems.

(b) Intact core technique: This method attempted to estimate rates of nitrification in intact sediment cores and therefore avoided the disruption of chemical and physical gradients established within the sediment deposits

which occurred when applying the slurry technique. The procedure was essentially that described by Hall (1984). The addition of the nitrification inhibitor to deep sediment layers was achieved using long narrow gauge syringe needles externally marked with a centimetre distance scale. This allowed the depth of needle penetration into the sediment to be accurately judged. All estimates of nitrification rates in the intact core systems were calculated using differences in ammonia concentrations. Incubation of littoral and profundal sediments were performed at *in situ* temperatures.

Physical and chemical measurements

Oxygen and temperature measurements were taken at metre depth intervals using a YSI (Yellow Springs Instruments) model 57 oxygen meter. All inorganic nitrogen analyses were as described by Hall (1984). Nitrogen gas was determined by Gas Liquid Chromatography using the procedures described in Jones *et al* (1980).

## Section 1 The effect of sample methodology on the estimation of nitrification potentials

### Introduction

Methods used to study the nitrification process in surface sediments can be broadly defined into two categories: (a) measurement of nitrate production under optimum conditions, the nitrification potential, in laboratory incubated sediment slurries (Chen et al., 1972; Isirimah et al., 1976; Cavari, 1977; Bostrum, 1981; Klapwijk and Snodgrass, 1982; Klingensmith and Alexander, 1983) and (b) independent estimates using nitrification inhibitors in sediment slurries (Vincent and Downes, 1981; Jones and Simon, 1981; Stewart et al., 1982) or intact sediment cores (Jones and Simon, 1981; Hall, 1984; Hall and Jefferies, 1984). With the exception of studies performed at the contractors laboratory all research on the freshwater sediment nitrification process have involved the use of sediment slurry techniques. The preparation of sediment slurries requires removal of discrete sediment layers and sample manipulation is usually facilitated by dilution. This procedure disrupts pre-formed concentration gradients within the sediments and interpretation of the results is made difficult by the ability of the lithotrophic nitrifiers to survive under adverse conditions. These organisms can contribute to the measured activity whilst not being active in situ. The range of conditions used to prepare slurries is shown in Table 2. It seemed opportune, therefore, to compare the effects of different slurry preparation procedures on the magnitude of the nitrification potential estimate. This would not only demonstrate the limitations in the use and interpretation of nitrification potential estimates but also allow a meaningful comparison to be made between different studies.

Materials and Methods

All samples were obtained from Grasmere lake and only the surface 1.0 cm of sediment used for slurry preparation. All comparisons are made against a standard measurement technique which was a 1:10 dilution (w/v) of sediment in overlying lakewater with substrate addition ($NH_3$-N as $(NH_4)_2SO_4$) equivalent to 10 mg N $l^{-1}$.

Results and Discussion

(a) The effect of diluent:-

The nitrification potential with lakewater as diluent was compared with that obtained using phosphate buffer (1 mM, pH 7.2) and a similar buffer with mineral salts added (Belser, 1977). The results for both littoral and profundal sediments are shown in Table 3 in conjunction with the effects of a light and dark sampling and incubation regime. The 'dark' samples involved wrapping the field sediment cores in aluminium foil immediately upon retrieval from the lake. Core manipulation was performed at ambient illumination but incubation was in flasks similarly wrapped in foil. Under the light conditions the buffer and defined medium gave similar nitrification potentials which were approximately double that estimated with lakewater as diluent. A similar pattern was observed under the dark regime but all the estimated potentials were approximately 25% greater. The reason why the potential estimates vary is still largely a matter of conjecture. Light, particularly at the blue region of the spectrum, has been shown to inhibit both the nitrite and ammonia oxidising bacteria although the former appear to be more sensitive (Hooper and Terry, 1974; Olson, 1981; Yoshioka and Saijo, 1984). The present results also indicate an inhibitory effect but as light intensities were not measured the results cannot be discussed in detail. The observations indicate that care

Table 2. Conditions of slurry preparations for sediment nitrification studies.

| Lake | Sediment Depth | Dilution (w/v) | Diluent | (Substrate) $N\ l^{-1}$ | Ref. |
|---|---|---|---|---|---|
| Erken | 0-1 | $2\text{-}6\times10^{-1}$ | Lakewater | 2 mg | Bostrum, 1981 |
| | 1-2 | | | | |
| Kinneret | Surface | $3\times10^{-2}$ | Lakewater | 2 mg | Cavari 1977 |
| Grasmere | 0-1 | $10^{-1}$ | Lakewater | 10 mg | Hall 1984 |
| Balgavies | 0-5 | $10^{-1}$ | Lakewater | zero | Christofi PhD thesis 1978 |
| Blelham | 0-5 | $10^{-1}$ | Lakewater | zero | |
| Blelham | Surface | $10^{-2}$ | Lakewater | 0.14 | Jones and Simon 1981 |
| Wisconsin Lakes | Surface | $5\times10^{-1}$ | Deionised water | zero | Chen et al. 1972 |
| Wingra | 0-10<br>10-20 | $5\times10^{-1}$ | Deionised water | 10 mg | Isirimah et al. 1976 |
| Ontario | Undefined | $5\times10^{-2}$ | Tapwater | 30 mg | Klapwijk & Snodgrass 1982 |
| Ilowa lakes | Undefined | Unknown | Defined medium | 2000 mg | Niewolak 1970 |

Table 3 Effect of diluent and illumination on nitrification potential.

Littoral sediments

| Diluent | Light | Dark |
|---|---|---|
| Overlying water | 39 | 56 |
| 1 mM $PO_4$ buffer (7.2) | 85 | 109 |
| *Defined medium | 89 | 122 |

Profundal sediments

| Diluent | Light | Dark |
|---|---|---|
| Overlying water | 34 | - |
| 1 mM $PO_4$ buffer | 69.8 | 85.5 |
| *Defined medium | 66.6 | 87.8 |

* 1 mM $PO_4$ buffer (7.2) + mineral salts all units µg N $g^{-1}$ dry wt $day^{-1}$.

must be taken during sample retrieval and incubation to obtain realistic nitrification potential estimates. The observed effect of the different diluents could possibly be related to the effect of phosphate buffer on the sediment exchange complex. Various nutrients or essential elements may be solubilised from the sediment complex and become available to the bacteria present in the sample. These may increase the observed activity due to increased growth rates or activities per cell.

(b) The effect of dilution interval.

The data shown in Table 2 indicate that a wide range of dilutions have been used in studies on nitrification in sediments. The highest dilutions represent an inoculation of sediment into a defined medium to enrich nitrifying bacteria. When estimating the nitrification potential it is important to measure the activity of bacteria present within the sample and not those capable of the most rapid growth under the laboratory conditions provided. Consequently a narrow range of dilutions were prepared and the results are presented in Table 4. These data are surprising in that the lower dilutions gave consistently lower nitrification potential estimates. The nitrifying bacteria (Kholedebarin and Oertli, 1977), and indeed the heterotrophic bacterial population (Jones, 1980), may be associated with the particles of sediment which would be present at a greater concentration in the low dilutions. The final section of this report provides some evidence that natural inhibitors of ammonia oxidising bacteria are present in sediments and it is tempting to speculate that increasing dilution removes the effects of these resulting in greater activity per cell.

(c) Effect of substrate concentration

The concentration of substrate in the diluted sediment slurry is dependent upon the ammonia present in the initial sample and the dilution interval. This

Table 4. Effect of dilution on nitrification potential (x(s))

Profundal sediments

| Dilution factor (w/v) | $\mu g\ NO_3\text{-}Ng^{-1}$ dry wt $day^{-1}$ |
|---|---|
| 1:2 | 26.3 (4) |
| 1:5 | 44 (3) |
| 1:10 | 46 (5) |
| 1:15 | 58 (8) |

Littoral sediments

| | |
|---|---|
| 1:2 | 4.06 (1) |
| 1:5 | 1.03 (1) |
| 1:10 | 9.97 (1) |
| 1:15 | 10.54 (2) |

Table 5. Effect of substrate concentration on nitrification potential

| [s] added (mg N $l^{-1}$ | $\mu g\ NO_3\text{-}Ng^{-1}$ dry wt $day^{-1}$ |
|---|---|
| 0 | 16 (6) |
| 10 | 52 (12) |
| 20 | 64 (11) |
| 50 | 49 (4) |

will obviously vary between sites but for comparative purposes it is essential that nitrification is not substrate limited during incubation. As the size of the ammonia pool in sediment deposits will vary on a seasonal basis it is possible that an ammonia limitation may occur at some times and not at others. Representative data are presented in Table 5 but it is important when using slurry activity for experimental data that appropriate controls with substrate additions are performed. Examples of this are presented in Section 4 of this report.

The effect of the different conditions on the magnitude of the nitrification potential indicates that there is little point in making direct comparisons between studies using different methodology. This is unfortunate as comparison of results between different environments can often provide indications of possible controlling mechanisms which could then be investigated by detailed study in a single environment. However, information on the effect of changing conditions, as shown in this section, does allow limited interpretation of other data. Using such an approach the available data suggest that nitrification rates are greater in eutrophic than in oligotrophic environments and that shallow eutrophic lake sediments support greater rates than profundal sediments of stratified eutrophic lakes (Chen et al., 1972; Christofi, 1981).

These results serve to illustrate that the nitrification potential estimate is very dependent upon the conditions of measurement and therefore care must be taken in their interpretation. The perturbation of the sample during handling has been recognised as being important by various workers. Correction factors are now applied to the measured potential to approximate better the in situ conditions (Henriksen et al., 1981; Belser and Mays, 1982; Cooper, 1984). Our results show that application of such factors must be undertaken with extreme caution.

Section 2. Zonal distribution of nitrifying activity within Grasmere lake and the relative importance of *in situ* and catchment derived sources of nitrate.

## Introduction

Nitrate concentrations in rivers show seasonal variations but are typically maximal during the winter months (Casey and Clarke, 1979; Nicholson, 1979; Stewart *et al.*, 1982). The maxima have been related both to temperature (Nicholson, 1979) and hydraulic load (Casey and Clarke, 1979; Stewart *et al.*, 1982; Hill, 1986). Similar seasonal cycles can be recognised in lakes. However, in the deeper lakes the winter peak of nitrate may not be the maximum concentration achieved during the year (Hall *et al.*, 1978). This indicates that the internal nitrate loading of lakes may, at times, be more important than that from the catchment. To accurately assess nitrate production within a lake the spatial, or zonal, distribution of rates of nitrate production must be determined. These distributions may vary on a seasonal basis and therefore, in order the describe the importance of nitrate production within lakes, the seasonal variation of nitrifying activity must be investigated.

## Results

### SEASONAL VARIATION OF NITRIFICATION

#### Sediments

Differences were observed in the seasonal cycle of nitrification rates in surface littoral and profundal sediments. The inorganic nitrogen analysis of incubated profundal sediment slurries indicated that only nitrite and not nitrate was produced. Corresponding estimates using intact core procedures indicated that nitrification rates were not detectable. This situation

persisted until March 1985 at which time the nitrifying ability of profundal sediments recovered and this was concommittant with rates being detectable in intact sediment cores. Upon detection of nitrate production in March the nitrification rates in slurries or intact cores did not change until the sediments became anaerobic in July (see Figure 2). Consequently the contribution of profundal sediments to the nitrate produced within the lake is restricted to the period March to July.

The seasonal cycle of nitrification in Grasmere littoral sediments is shown in Figure 3; data from two years are included for comparative purposes. It is now becoming clear that year to year variability of microbial activities in sediments is large but for the purposes of calculating the total nitrate produced by littoral sediments it was considered that the measured values were representative. Generally the rates of nitrification tended to be maximal when temperatures were at their highest. For ease of presentation the data in Figure 3 represent the rates from intact cores only. A full summary of the temperature corrected rates measured in sediment slurries is shown in Table 6. From these data it was clear that there were differences in the two rate estimates.

Water

To preserve clarity only data from representative depths of the water column are presented in Figure 4 along with the associated ammonia and nitrate concentration. The 5 m depth was not entirely representative of surface water as rates in water samples obtained with surface dips were always lower than those in samples from 5 m. However the 15 m depth was representative for hypolimnetic water at 10 m and 20 m depth. At 5 m depth a peak of activity was noted in May associated with a spring diatom bloom which was followed by activity being undetectable until lake overturn in late October. It was also

Table 6. Potential nitrification rates in the surface 1.0 cm of littoral and profundal sediments of Grasmere

| | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | June | July | Au |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LITTORAL* | 64 | 61 | 60 | 68 | 81 | 70 | 57 | 69 | 85 | 111 | 40 |
| PROFUNDAL* | An | $NO_2$ | $NO_2$ | $NO_2$ | $NO_2$ | $NO_2$ | 35 | 32 | 37 | 35 | An | An | An |

* µg N $g^{-1}$ dry wt $day^{-1}$

An = Anaerobic

$NO_2$ = $NO_2$ produced

apparent that in surface water it was not always possible to relate nitrifying activity to changes of inorganic nitrogen concentrations, particularly during the spring diatom bloom when nitrogen assimilation rates were high. Nitrification rates in the hypolimnion, represented in Figure 4 by 15 m depth, were generally greater than those in surface water. Activity was observed in May which again coincided with the spring diatom bloom. A second peak of activity was observed in July which was associated with increasing nitrate concentrations typical of nitrification. The hypolimnion was then depleted of oxygen and most of the nitrate produced was removed from the water column. Upon lake overturn (in October) ammonia rich water was oxygenated which resulted in maximum nitrifying activity being detected.

## Discussion

Prior to the present work there had been few detailed studies of the seasonal variability of nitrification in lakes, however, the trends noted in the present work have also been identified in less detailed studies. The initial increase in activity of the water column in the early stages of stratification has been observed in eutrophic Scottish lochs (Stewart et al., 1977) and may have been associated with spring diatom blooms. Similarly other workers have observed maximal nitrifying activity at overturn or during the circulation period (Cavari, 1977; Robarts et al., 1982; Bostrum, 1981). This has been reported to be due to the mixing of ammonia-rich hypolimnetic water with aerobic surface water (Cavari, 1977) and/or wind induced turbulence eroding surface sediments to provide an inoculum in the water column (Bostrum, 1981). The phase of nitrification in hypolimnetic water is a regular occurrence in Grasmere (Hall et al., 1978; Hall and Jefferies, 1984) and in many other lakes (Christofi et al., 1981; Takahashi et al, 1982; Yoshioka and

Saijo, 1985; Tezuka, 1984; Brezonik, 1972; Larsen, 1977). It should be noted that 75% of the nitrate formed during this period in Grasmere lake was dissimilated as the hypolimnion became deoxygenated. The peaks of activity noted early in stratification and at overturn, particularly in surface water, result in nitrate being made available and do not facilitate nitrogen loss from the system.

As with lakes and other freshwaters in general there have been few studies on the seasonal cycles of nitrification in sediments. Stewart et al (1977) reported a peak of activity in July for Loch Balgavies sediments. This is a shallow lake which rarely stratifies and the sediments, therefore, would be similar to the littoral sediments of Grasmere. These also show a peak of activity in mid-summer which coincides with maximum water temperatures. Available data on profundal sediment deposits are contradictory with both maximal (Bostrum, 1981) or minimal (Cavari, 1977) rates being reported at overturn. Both the reported studies, however, used enrichment techniques and therefore the results cannot be compared with the more reliable short term incubation studies used here. The absence of nitrification in profundal sediments after lake overturn has not been previously reported.

NITRATE LOAD TO THE LAKE:

Nitrate concentrations in river waters are typically maximal in mid-winter, however, as shown in Figure 5, the river Rothay did not show this pattern over the study period. The months of January and February were drier than average with an observed rainfall of 318 mm compared with an expected 421 mm (FBA unpublished data) which may account for the observations (nitrate concentration data for other years have shown the expected winter maximum (Fig. 5). The river flows and nitrate concentrations varied by approximately an order of magnitude during the study indicating that both could equally affect the nitrate load to the lake.

The seasonal variation of nitrate production in the water column and sediments of Grasmere is shown in Table 7. The water contribution is the sum of surface and hypolimnetic values whilst the sediment contribution is divided into values calculated from the nitrification potential and those obtained from measurements on intact cores. The activity in the water column in October and November is found throughout the depth profile and is associated with lake circulation. The activity in May is largely confined to surface water and is associated with increasing temperatures. The nitrate produced during July and August is due entirely to the hypolimnetic phase of nitrification. These periods are separated by little activity in the water column due possibly to low temperatures (December - April), ammonia limitation in surface water (June - November) and deoxygenation of hypolimnetic water (September - November). The data in Table 7 show that on average the nitrate load calculated from slurry derived rates was 5.9 times greater (range 1.6-11.2) than that estimated from intact core techniques. This discrepancy in estimated rates was surprising as reasonable agreement between two similar approaches has been reported for marine sediments (Henriksen et al., 1981). However, in the

Table 7. The monthly nitrate load (in kg $NO_3$-N) in the water column and sediments

N.B. sediment values calculated from (a) nitrification potentials (b) in

| | | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | June |
|---|---|---|---|---|---|---|---|---|---|---|
| Sediments | Potential[a] | 514 | 475 | 490 | - | 855 | 626 | 723 | 247 | 503 |
| | Intact core[b] | 115 | 88 | 57 | - | 90 | 85 | 144 | 30 | 45 |
| Water | | 458 | 279 | 2 | - | 0 | 2 | 0 | 1212 | 0 |

absence of a definitive method to determine rates of nitrification in sediments it is difficult to decide which estimate is the more reliable. In an attempt to rationalize these data a tentative nitrate budget was calculated for the circulation period (October - March) when problems associated with incomplete mixing of heterogeneous water masses were eliminated. Such an approach was considered valid as (i) the retention time of the lake is sufficiently short, (ii) the sites used to obtain the data were considered to be representative of the whole lake and (iii) reliable estimates of nitrogen assimilation and denitrification could be obtained from previous work. The budget calculations are shown in Table 8 which indicates that if the nitrate production within the lake is calculated, including data on the sediment nitrification potential (slurry data), an unaccountable surplus of nitrate is obtained. If data obtained using intact cores are used the budget balances very well. This suggests that the intact core method provides the most reliable estimates of nitrification rates in sediments.

Assuming that the reliable sediment data is provided by the intact core rate measurements the monthly values for internal and external nitrate loads to Grasmere are shown in Table 9. On an annual basis the inflow accounts for 157 kg $NO_3$-N $ha^{-1}$ whilst the internal load accounted for 80 kg $NO_3$-N $ha^{-1}$. However there were strong seasonal influences on the relative importance of internal and external nitrate loads. Comparison of the data shown in Tables 7 and 9 show that when the internal nitrate load is important in the total nitrate input to the lake it is due to nitrifying activity in the water column rather than that in the sediments.

## Discussion

The nitrifying activity in the water column which was significant in the nitrate budget of Grasmere can be identified as (a) the early season activity noted in both surface and deeper water, (b) the nitrification phase which

Table 8. Nitrate budget calculation for Grasmere lake (Oct-March)

(kg $NO_3$-N)

| | Source | Sink |
|---|---|---|
| Inflow | 6721 | |
| Outflow | | 7280 |
| Rainfall | 227 | |
| Nitrate from lake | 1251[a] | |
| | 4375[b] | |
| Denitrification | | 1096 |
| Assimilation | | 244 |
| Total | 8199[a] | 8620 |
| | 11,323[b] | 8620 |

(a) calculated using sediment intact core data

(b) calculated using sediment nitrification potential data

ab values for January calculated as x of December and February.

Table 9 Monthly nitrate load from the inflow to Grasmere and nitrate produced wi
percentage of the total nitrate load to the lake (kg $NO_3$-N)

| | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | June | July | Aug |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Inflow Load | 1817 | 660 | 756 | 1156 | 1089 | 1243 | - | 347 | 456 | 673 | 682 |
| Lake Load | 573 | 367 | 59 | - | 90 | 87 | 144 | 1242 | 45 | 1246 | 730 |
| Lake Load as a % of Total | 24 | 36 | 7 | - | 8 | 7 | - | 78 | 9 | 65 | 52 |

occurs only in the hypolimnion and (c) the activity after overturn. Most authors conclude that the sediments are the most important sites of nitrification based either on viable counts of nitrifier populations (Tezuka, 1985) or measured nitrification rates (Chen et al., 1972; Isirimah et al, 1976; Vincent and Downes, 1981). However, in drawing such conclusions a precise definition of importance is required. If only the surface 1.0 cm of sediment remains aerobic (probably overestimated) then the mean sediment nitrification rate (on a volume basis) must exceed that in the water column by 780 fold before they become the most important site of nitrification in Grasmere. In simple terms, the water column sustains low rates of nitrification when compared with the rates measured in sediments but the greater active volume of water makes this site more important in whole lake nutrient budgets. However, in terms of nitrogen removal from the lake system the sediments may be the more important site due to coupled nitrification and denitrification sequences across a permanent redox boundary. Such an assumption may not be valid for deep water sediments if the absence of nitrification observed in Grasmere profundal sediments is observed in comparable system.

## Section 3. The effect of eutrophication on the nitrification process

### Introduction

The nitrification process has been investigated in Arctic (Schell, 1974), Antarctic (Vincent et al., 1981) and warm lakes (Cavari, 1977). Most work has, however, involved lakes of temperate climatic regions and data are available on shallow lakes (Stewart et al., 1977), stratified oligotrophic (Vincent and Downes, 1981), mesotrophic (Hall et al., 1978) and eutrophic (Christofi et al., 1981) lakes including hardwater and softwater examples (Chen et al., 1972). This variety of habitats is further matched by the variability of the methodology employed for the bacteriological investigation. As slight changes in methodology can greatly affect the activities estimated it is difficult to compare results between studies more than observing general trends. It was logical therefore to extend the present studies and investigate lakes of differing degrees of eutrophication. Such studies would provide a database from which the effects of changing land use on a particular water body may be better able to be assessed and aid in water management policies. Moreover, comparison between different environments may produce information on the underlying control of the nitrification process.

### Results and Discussion

#### Water column

In all the lakes studied a water sample from a 5 m depth was considered to be representative of epilimnetic water. The ammonia and nitrate concentrations and the corresponding estimates of nitrification rates are shown in Figure 6. The surface waters of lakes are influenced by inflows to the lake system. In summer these inflows tend to be warmer than the deep lake water and therefore

selectively mix with the epilimnetic or metalimnetic water volumes. The inflows can affect water chemistry as well as providing an inoculum of bacteria. The observed high viable counts of heterotrophic bacteria (Jones, 1979) as well as increased nitrifying activity (Cavari, 1977) have been attributed to such processes. In addition wind-induced turbulence can cause resuspension of shallow water sediments which could contribute to surface water activity. The different geographical locations of the lakes sampled in the present study might result in different localised climatic conditions, and therefore any observed differences may not be due to increasing eutrophication. Given the dynamic condition of the nitrogen cycle in surface waters it is clear that interpretation of these data may create problems. For these reasons the main discussion of the present results will focus on the process of nitrification in the hypolimnion and sediments, which are less likely to be influenced by atmospheric climatic conditions.

Evidence for nitrification occurring in the hypolimnia of all lakes except oligotrophic Buttermere was obtained from the change of ammonia and nitrate concentrations between sampling visits. In most cases the increases of nitrate concentrations were associated with nitrifying activity being observed in laboratory incubated water samples. These results are shown in Figure 7. The only occasion when nitrifying activity was not associated with increased nitrate concentration was in the eutrophic Blelham tarn. However, in eutrophic lakes nutrient pools can change quite rapidly and the present sampling frequency of four weeks may not have given sufficient resolution to detect transient peaks of activity. The maximum nitrate concentration for example, may have occurred prior to sampling, and therefore may have been decreasing at that moment although the trend from the previous sampling date was an apparent increase in concentration. The appearance of nitrate only indicates that nitrification has occurred sometime in the immediate past.

The rates of nitrate production tended to increase with increasing eutrophication of the water bodies. In the oligotrophic lake, Buttermere, with the exception of one sample taken under very windy conditions in May, the observed rates were less than 10 μg N $l^{-1}$ $day^{-1}$ with a mean value of 4 μg N $l^{-1}$ $day^{-1}$. The corresponding figures for the mesotrophic lake were 0-40 μg N $l^{-1}$ $day^{-1}$ with a mean value of 14.0 μg N $l^{-1}$ $day^{-1}$ and for the eutrophic lake, range 0-180 μg N $l^{-1}$ $day^{-1}$ with a mean of 47 μg N $l^{-1}$ $day^{-1}$.

Sediments

Using both intact core and slurry techniques nitrifying activity was measured in the surface 1.0 cm of littoral and profundal sediments of the three main lakes sampled in this study. The results are presented on Table 10, as mean values to improve clarity, the standard deviations are not presented but a rough approximation can be obtained using ± 10%.

In Grasmere lake, initially selected as a mesotrophic lake for this study, the discharge of sewage effluent into the hypolimnion had significantly increased the hypolimnetic areal oxygen deficit and therefore the profundal sediments must be regarded as being characteristic of eutrophic lakes. In the present study the profundal sediment slurries only produced nitrite (as had been observed after overturn in the previous year) and this continued until deoxygenation of the hypolimnion. Therefore the recovery of nitrate production, observed in the previous year, did not occur. Concommitantly rates of nitrification were not detected using intact core methods. However, data from the previous year (when nitrification was active), shown in Table 10 along with the present data, indicated that rates of nitrification were greater in profundal sediments than littoral sediments. Also the rates in littoral sediments were greater than those in the oligotrophic lake whilst rates in profundal muds were unchanged by eutrophication.

In Esthwaite, the eutrophic lake, rates of nitrification in littoral sediments were greater than those in the profundal muds. Moreover, the rates in littoral sediments were greater than those observed at similar sites in the mesotrophic lake. Surprisingly, although the number of observations were limited, rates in profundal sediments were not significantly different from those in the oligotrophic or mesotrophic environments.

In summary, rates of nitrification in littoral sediments increase with eutrophication irrespective of the method used. The rates in profundal sediments do not increase with eutrophication and in some lakes, possibly those with the greatest hypolimnetic areal oxygen deficit, the nitrification process appears to become uncoupled with ammonia oxidation only proceeding to nitrite. In the oligotrophic environment there appears to be little difference in rates of nitrification between littoral and profundal sediments. To confirm these general trends other lakes were sampled and the results are shown in Table 11. Derwentwater is classified as oligotrophic and correspondingly there was no significant difference between rates of nitrification in sediment slurries from littoral or profundal sites. Blelham tarn was sampled as a eutrophic lake and the profundal sediments produced mostly nitrite although some nitrate was formed. However the nitrification rates in littoral sediments were greater than those in profundal muds. Elterwater lake was selected as a highly eutrophic system and correspondingly the profundal muds (7.5 m) produced only nitrite. The production of nitrite by sediment slurries has been investigated further in Section 4 of this report.

The average extractable ammonia concentration in the sediments of various lakes is shown in Table 12. In the littoral sediments the increasing eutrophication is correlated with increasing ammonia concentrations, which in turn must support a greater population of nitrifying bacteria, i.e. the

carrying capacity of the environment increases with increasing eutrophication. However, within a particular lake the seasonal cycle of nitrification correlates more closely with temperature rather than extractable ammonia concentrations regardless of degree of enrichment. In profundal sediments ammonia concentrations are generally greater than in littoral deposits but these are not matched with increasing nitrification. Furthermore increasing ammonia concentrations associated with increasing eutrophication is not associated with increased nitrification; in fact in the sediments with the greatest concentration of ammonia nitrification appears to become uncoupled as nitrite accumulates. Therefore it is likely that ammonia concentration is not the major factor controlling the carrying capacity of nitrifiers in profundal sediments.

It is unlikely that the high ammonia concentrations in the profundal sediments of Grasmere and Esthwaite are inhibitory to the nitrifiers as has been demonstrated in laboratory cultures (Anthonisen et al., 1976). This can be stated with some confidence as laboratory experiments, with both the profundal sediments of Buttermere and the littoral sediments of all the lakes, indicated that activity in these deposits was unaffected by added ammonia concentrations far in excess of those measured in Grasmere and Esthwaite profundal sediments.

Table 11. Rates of nitrification in lakes used for comparative purposes

| Date | Lake | Profundal* | | Littoral* | |
|---|---|---|---|---|---|
| | | $NO_2$-N | $NO_3$-N | $NO_2$-N | $NO_3$-N |
| 14/5 | Derwentwater (O) | - | 26 | - | 22 |
| 1/5 | Blelham (E) | 36 | 56.6 | - | 84.8 |
| 4/5 | Elterwater (HE) | 14.8 | - | - | ND |

* all rates in µg N $g^{-1}$ dry wt. $day^{-1}$

O = oligotrophic

E = eutrophic

HE = hypereutrophic

ND = Not Done

## Section 4 Unusual patterns of nitrification associated with the profundal sediments of very productive lakes

### Introduction

Section 3 of this report discusses the observed differences between rates of nitrification in the littoral and profundal sediments of different lakes. This demonstrated that as lakes become more productive rates of nitrification in littoral sediment deposits become more active, possibly in response to increasing ammonia concentrations. However, the corresponding activity in profundal deposits showed little change with lake productivity despite a similar trend of increasing ammonia concentrations. Moreover, in the profundal deposits of very productive lakes, work with sediment slurries indicated that substantial concentrations of nitrite could be produced upon aerobic incubation. This was also true of profundal sediments in Grasmere lake which although classified as a mesotrophic lake has a sewage effluent discharged directly into the hypolimnion. This method of effluent disposal started during this study and an immediate increase in the hypolimnetic areal oxygen deficit from approximately 350 to 510 mg $O_2$ $m^{-2}$ $day^{-1}$ was noted. Production of nitrite by profundal sediment slurries had been observed before the sewage effluent was diverted into the lake but subsequently complete nitrification, as evidenced by nitrate production, was noted to recover (see Section 1). Such a recovery process was not seen after the effluent discharge into the hypolimnion. The profundal sediments of eutrophic lakes are anaerobic for the period May to November and consequently would not support nitrification. This study was restricted, therefore, to the lake circulation period from December to April. Also at this time the water column was isothermal and all sediments were at the ambient water temperature.

Results and Discussion

Rates of nitrite and nitrate production by littoral and profundal sediment slurries from Blelham Tarn, Esthwaite Water and Grasmere lake are shown in Table 13. These results confirm previous observations and the present discussion will only concern the production of nitrite by profundal sediments of Blelham and Grasmere. The data on Table 13 show a decrease of nitrate concentration associated with nitrite production. In these mixed systems it was not possible to control the availability of different nitrogen substrates (ammonia present in sediments, nitrate present in lakewater). However, under more closely controlled laboratory systems it was demonstrated (see Table 14) that the nitrite produced by Blelham and Grasmere profundal sediments was due to nitrate reduction rather than ammonia oxidation and, moreover, that nitrification could not be detected. These data also show that nitrification was occurring in the littoral sediments of these lakes. For comparative purposes the response of the profundal sediments of Esthwaite, which supported nitrification, are also shown. All sediments in which nitrification was occurring responded in a predictable fashion to changing substrate ($NH_3$-N) concentrations.

These observations immediately raised the question as to why nitrification was absent from the profundal sediments of some eutrophic lakes despite the presence of an oxidised sediment water interface which, in theory, was an ideal environment for nitrifying bacteria. It was possible that any nitrate produced could be denitrified to nitrogen gas and therefore to examine this possibility the closed sediment slurry systems (see Section 1) were developed. Representative results are shown in Table 15. The absence of nitrogen gas production after the addition of ammonia to Grasmere profundal sediments confirms the absence of nitrification from these deposits. The addition of nitrate resulted in nitrite production (as noted before) and also stimulated nitrogen gas production.

Table 13 Rates of nitrate and nitrite production in sediment slurries from three productive lakes.

| | PROFUNDAL | | LITTORAL | |
|---|---|---|---|---|
| | $NO_2$-N | $NO_3$-N | $NO_2$-N | $NO_3$-N |
| ESTHWAITE | ND | 48(5) | ND | 201(24) |
| GRASMERE | 26(4) | (-37) | ND | 99(11) |
| BLELHAM | 58(3) | (-39) | ND | 139(4) |

all units µg N $g^{-1}$ dry wt. $day^{-1}$ x(s)

ND = Not detectable

Table 14. The effect of adding ammonia (10 mg N $l^{-1}$) and nitrate (10 mg N $l^{-1}$) to sediment slurries from Grasmere, Blelham and Esthwaite. (+ ATU means the addition of the nitrification inhibitor Allylthiourea). All units in µg N $g^{-1}$ dry wt. $day^{-1}$

**(a) GRASMERE**

| PROFUNDAL | $NO_2$-N | $NO_3$-N |
|---|---|---|
| + $NH_3$ | 3 | 1 |
| + $NO_3$ | 20 | -18 |
| + $NO_3$ + ATU | 18 | -18 |

| LITTORAL | $NO_2$-N | $NO_3$-N |
|---|---|---|
| + $NH_3$ | 0 | 117 |
| + $NO_3$ | 0 | 27 |
| + $NO_3$ + ATU | 0 | 5 |

**(b) BLELHAM**

| PROFUNDAL | $NO_2$-N | $NO_3$-N |
|---|---|---|
| + $NH_3$ | 0 | -12.8 |
| + $NO_3$ | 65 | Not Done |

| LITTORAL | $NO_2$-N | $NO_3$-N |
|---|---|---|
| + $NH_3$ | 0 | 152 |
| + $NO_3$ | 0 | 129 |

**(c) ESTHWAITE**

| PROFUNDAL | $NO_2$-N | $NO_3$-N |
|---|---|---|
| + $NH_3$ | - | 122 |
| + $NO_3$ | - | 81 |

Additional evidence for the inhibition of nitrification in some profundal sediments was provided by the addition of 3 g profundal sediment to nitrifying littoral sediments from the same lake. This addition resulted in a decrease in the rate of nitrate production which could not be explained by stimulation of denitrification. Moreover, the data shown in Table 16 indicate that the inhibitory components were not removed by autoclaving the profundal sediment.

In an attempt to determine the nature of the inhibitory component Grasmere profundal sediments were stored under aerobic conditions at 10°C and 20°C and under anaerobic conditions at 20°C. At periodic intervals aliquots of these stored sediments were removed and the degree of inhibition of nitrification in freshly sampled littoral sediments determined. The results of a typical experiment in which sediment was stored for 9 days is shown in Table 17. These data indicate that storage under aerobic conditions rapidly removed some of the inhibitory property with the degree of inhibition of nitrification in littoral sediments decreasing from approximately 80% to 50% within 48 h. This level of inhibition was subsequently maintained. Storage under anaerobic conditions maintained the high level of inhibition noted in the freshly sampled material.

Table 15 The effect of adding ammonia (10 mg N $l^{-1}$) and nitrate (10 mg N $l^{-1}$) on ammonia, nitrite, nitrate and nitrogen gas production in Grasmere profundal sediment slurries (all units µg N $g^{-1}$ dry wt. $day^{-1}$)

| | $NH_3$-N | $NO_2$-N | $NO_3$-N | $N_2$-N |
|---|---|---|---|---|
| + $NH_3$ | -2.1 | 0 | 0 | 0 |
| + $NO_3$ | +6.4 | +7.4 | -25.3 | +11.4 |

Table 16 The effect of adding 3.0 g profundal sediment on nitrate production in littoral sediment slurries

| | LITTORAL* | LITTORAL + 3.0 g PROFUNDAL* | + 3.0 g PROFUNDAL (AUTOCLAVED) |
|---|---|---|---|
| GRASMERE | 93 (7) | 44 (5) | ND |
| | 94 (9) | 24 (6) | 20 (1) |
| BLELHAM | 227 (13) | 39 (8) | ND |

* $\mu g\ N\ g^{-1}$ dry wt. $day^{-1}$ x (s)

ND = Not done

Table 17 Effect of storage of Grasmere profundal sediments on the inhibition of nitrification in Grasmere littoral sediments

| DAYS STORAGE | CONTROL[a] | 10°C AEROBIC[b] | 20°C AEROBIC[b] | ANAEROBIC[b] |
|---|---|---|---|---|
| 0 | 94 (9)* | 25 (7) | 25 (7) | 25 (7) |
| 2 | 103 (11) | 59 (2) | 49 (7) | 24 (2) |
| 7 | 95 (2) | 51 (6) | 58 (3) | 18 (4) |
| 9 | 104 (9) | 57 (10) | 53 (6) | 15 (5) |

* all units in μg $NO_3$-N $g^{-1}$ dry wt. $day^{-1}$

[a] freshly sampled littoral sediments

[b] the effect of adding 3.0 g stored sediment

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